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(54) Title: STRESS PROTEINS AND USES THEREFOR

(57) Abstract

Stress proteins and their use to immunize an individual against a nonviral infection or to induce immune tolerance in an individual, as well as a method of immunizing an individual by administering a selected stress protein and a method of inducing immune tolerance in an individual by administering a selected stress protein.

STRESS PROTEINS AND USES THEREFOR

Description

Background of the Invention

Although the function of stress proteins is not entirely clear, it appears that some participate in assembly and structural stabilization of certain cellular and viral proteins, and their presence at high concentrations may have an additional stabilizing effect during exposure to adverse conditions. Neidhardt, F.C. and R.A. VanBogelen, In: Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology, (eds. Neidhardt, F.C., Ingraham, J.L., Low, K.B., Magasanik, B. Schaechter, M. and Umbarger, H.E. (Am. Soc. Microbiol., Washington, D.C.), pp. 1334-1345 (1987); Pelham, H.R.B. Cell, 46:959-961 (1986); Takano, T. and T. Kakefuda, Nature, 239:34-37 (1972); Georgopoulos, C. et al., New Biology, 239:38-41 (1972). Phagocytic host cells produce a hostile environment for foreign organisms, and the ability to produce stress proteins has been implicated in the survival of bacterial pathogens within macrophages Christman, M.F. et al., Cell, 41:753-762 (1985).

Mycobacterium (M.) tuberculosis and Mycobacterium (M.) leprae are the etiologic agents of tuberculosis and leprosy, respectively. These diseases afflict 20-30 million people and continue to present a significant global health problem. Joint International Union Against Tuberculosis and World Health Organization Study Group, Tubercle, 63:157-169 (1982); Bloom, B. and T. Godal, Rev. Infect Dis. 5:765-780 (1983). To develop more effective

tools for the diagnosis and prevention of these diseases, it is important to understand the immune response to infection by mycobacterial pathogens.

The antibody and T-cell responses to infection or inoculation with killed mycobacteria have been studied in humans and in animals. Human patients with tuberculosis or leprosy produce serum antibodies directed against at least 12 mycobacterial proteins. Some of these proteins are also recognized by well-characterized murine monoclonal antibodies. Mice immunized with mycobacterial lysates produce antibodies that are directed predominantly to six M. tuberculosis and six M. leprae protein antigens. Engers, H.D., Infect. Immun., 48:603-605 (1985); Engers, H.D., Infect. Immun., 51:718-720 (1986). Genes encoding these 12 mycobacterial antigens have been cloned, and recombinant proteins produced from these clones have been used to investigate the human T-lymphocyte response to mycobacterial infection. Husson, R.N. and R.A. Young, Proc. Natl. Acad. Sci., USA, 84:1679-1683 (1987); Young, R.A. et al., Nature, 316: 450-452 (1985); Britton, W.J. et al., Lepr. Rev., 57, Suppl. 2, 67-75 (1986).

Protection against mycobacterial disease involves cell-mediated immunity. Joint International Union Against Tuberculosis and World Health Organization Study Group, Tubercle, 63:157-169 (1982); Hahn, H. and S.H.E. Kaufman, Rev. Infect. Dis., 3:1221-1250 (1981). T lymphocytes cloned from patients or from volunteers immunized with killed mycobacteria have been tested for their ability to recognize the recombinant mycobacterial proteins. Lymphocyte-proliferation assays demonstrate

that most of the antigens identified with monoclonal antibodies are involved in the T-cell response to mycobacterial infection or vaccination in mice and in humans. Limiting dilution analysis indicates that 20% of the mycobacterial-reactive $CD4^+$ T lymphocytes in mice immunized with M. tuberculosis recognize a single protein, the 65-kDa antigen. Kaufman, S.H.E. et al., Eur J. Immunol., 17:351-357 (1987).

Summary of the Invention

The present invention relates to stress proteins and methods of modulating an individual's immune response, either to a pathogen or to his or her own cells, such as occurs in autoimmune diseases. In particular, it relates to the use of such stress proteins as a "vaccine" in immune prophylaxis therapy, which results in an induction or enhancement of immune response to a selected pathogen and as an immunotherapeutic agent in treatment of autoimmune diseases, which results in a decrease of an individual's response to his or her own cells. In immune prophylaxis, stress proteins are administered to prevent or reduce the effects in an individual of a pathogen, which can be any virus, microorganism or other organism or substance (e.g., a toxin or toxoid) which causes disease. In preventing or reducing adverse effects of nonviral pathogens (e.g., bacteria, mycobacterial) according to the method of the present invention, an individual's immune response to the nonviral pathogen's stress protein(s) is induced or enhanced through the administration of a vaccine which includes the pathogen's stress protein(s) and, generally, an adjuvant.

Preventing or reducing adverse effects of viral pathogens, as well as preventing cell transformation or reducing the extent to which it occurs, according to the present method, is effected by transiently enhancing an individual's immune surveillance system. In this instance, the causative pathogens (i.e., virus; transforming agent) do not have stress proteins of their own. Enhancement of immune response can be effected by modulating the immune cells by stimulation with a nonviral stress protein (e.g., a bacterial stress protein) or modulating the individual's stress response by any means (e.g., local application of heat).

In immune therapy, such as is used in treating autoimmune diseases, stress proteins known to be involved in the autoimmune response are administered to turn down an individual's immune response by tolerizing the individual to the stress proteins. Alternatively, the immune response to stress protein, which is known to occur in autoimmune disease, is reduced by interfering with the ability of immune cells which respond to stress proteins to do so.

A selected stress protein of the present invention can be administered to an individual, according to the method of the present invention, and result in an immune response which provides protection against subsequent infection by a nonviral organism (e.g., bacteria, mycobacteria, other infectious agents which produce stress proteins). Alternatively, a selected stress protein can be administered to an individual, generally over time, to induce immune tolerance against the selected stress protein. For example, a selected stress

protein can be administered in multiple doses over time in order to induce immune tolerance against an autoimmune disease such as rheumatoid arthritis.

Brief Description of the Drawings

Figure 1 is a graphic representation of the homologies between mycobacterial antigens and known stress proteins. Figure 1A is a representation of sequence similarity between portions of the M. tuberculosis 71-kDa antigen (residues 1-204; TB 71kDa) and the E. coli DnaK protein (residues 430-469). Figure 1B is a representation of sequence similarity between portions of the M. tuberculosis 65-kDa antigen (residues 1-540; TB 65kDa) and the E. coli GroEL protein (residues 1-547).

Figure 2 is a comparison of the amino acid sequence of the human P1 protein (573 residues) and the amino acid sequence of the groEL protein (547 residues).

Figure 3 is a comparison of the amino acid sequence of the human P1 protein (573 residues), which is a homolog of groEL protein, and the amino acid sequence of the 65kDa M. leprae protein (540 residues).

Figure 4 is a comparison of the amino acid sequence of the human P1 protein (573 residues), which is a homolog of the groEL protein, and the amino acid sequence of the 65kDa M. tuberculosis protein (540 residues).

Detailed Description of the Invention

The present invention is based on the observation that stress proteins are among the major antigens available for presentation to T lymphocytes and may be common immune targets in a broad spectrum of infectious

diseases. Immune responses to stress proteins are involved in immune surveillance by the body and a variety of different T cell types has been shown to recognize highly conserved stress protein determinants. Several observations, described below, suggest a model of immune surveillance in which self-reactive T cells provide a first line of defense against infection or other invasion by pathogens and against cell transformation by recognizing and helping to eliminate stressed autologous cells, as well as cells infected with intracellular bacteria. Without wishing to be bound by this model, it is presented as one means by which it is possible to explain why prokaryotic and eukaryotic cells respond to a variety of potentially damaging stimuli, such as elevated temperature, by increasing the synthesis of a family of proteins, referred to as stress proteins, which are among the most highly conserved and abundant proteins found in nature.

Investigation of antigens involved in the immune response to the tuberculosis and leprosy bacilli (M. tuberculosis and M. leprae) initially led to the observation that a variety of stress proteins are among the major targets of the immune response, as is described at greater length below.

Further assessment has demonstrated that stress proteins may be common immune targets in a broad spectrum of infectious diseases. Sequence analysis has revealed 70-kDa heat shock protein homologues among major antigens of the protozoan parasites Plasmodium falciparum (Bianco, A.E. et al., Proc. Natl. Acad. Sci. USA, 83:8713-8717 (1986)) and Schistosoma mansoni (Hedstrom, R. et al., J.

Exp. Med., 165:1430-1435 (1987)) and the malarial parasite Brugia malayi (Selkirk, M.E. et al., J. Cell Biochem., 12D:290 (1988)). Similarly, homologues of GroEL have been found among antigens involved in the immune response to Salmonella typhimurium and Coxiella. Vodkin, M.H. and J.C. Williams, J. Bacteriol, 170:1227 (1988). The presence of stress proteins among major immune targets in a variety of human pathogens is support for the idea that the stress response may be a general component of infection and that stress proteins should be considered among candidates for subunit vaccines. All organisms respond to heat by inducing synthesis of heat shock proteins (hsps), which are a group of proteins. This response is the most highly conserved genetic system known and has been shown to occur in every organism, including microorganisms, plants and animals, investigated to date. Many of the characteristics of the response are common to all organisms and the hsps are among the most highly conserved proteins known. For example, hsp90 family and hsp70 family proteins are present in widely diverse organisms. The proteins in each family--even in such diverse organisms--show approximately 50% identity at the amino acid level and at the nonidentical residues, exhibit many similarities. Several of the proteins induced by heat are also induced by a variety of other stresses. The hsps or a closely related/similar protein are present in all organisms at normal temperatures and have been shown to have key functions in normal cell metabolism. Lindquist, S. and E.A. Craig, Ann. Rev. Genet., 22:631-677 (1988). Because the stress response is common to prokaryotes and

eukaryotes and stress proteins are among the most highly conserved in sequence, it is reasonable to expect that an antigen from one pathogen could immunize against another pathogen. Exposure to foreign stress proteins early in life might, in fact, induce a degree of immunity to a variety of infectious agents. If so, this could provide an explanation for the observation that, for many pathogens, only a fraction of infected individuals actually acquire clinical disease.

The following is a description of the relationship which has been observed between stress proteins and the immune response to mycobacterial infection; of the observation and supporting information that stress proteins are immune targets in many non-viral infections; of recognition of the fact that immune responses to conserved stress protein determinants may play an important role in autoimmune pathology in rheumatoid arthritis, as well as in adjuvant arthritis; and of the role of stress proteins in immune surveillance, as well as a model proposed for immune surveillance in which self-reactive T cells provide a first line of defense against infection and cell transformation.

Mycobacterial Stress Proteins are Targets of the Immune Response

An intriguing relationship between stress proteins and the immune response to mycobacterial infection has been observed. A more detailed examination of stress protein determinants and immune response mechanisms is essential to understanding the relationships among stress proteins, infection, and immunity.

In view of the involvement of proteins of M. tuberculosis and M. leprae in humoral and cell-mediated immune responses and to establish the functions of the these proteins in the mycobacterial cell, the DNA encoding several of the M. tuberculosis and M. leprae antigens have been sequenced. It has been demonstrated, as a result, that many of these mycobacterial protein antigens exhibit striking sequence similarity to known stress-induced proteins. Three of the M. leprae and two of the M. tuberculosis protein antigens studied have been shown to exhibit striking sequence similarity to known stress proteins. For reasons discussed in the Exemplification, it is concluded that two of the M. leprae and two of the M. tuberculosis antigens are homologues of the E. coli DnaK and GroEL proteins.

In experimental mice, immunization with mycobacterial lysates elicits antibody responses to at least six M. tuberculosis protein antigens and a similar number of M. leprae protein antigens. Monoclonal antibodies specific for these proteins have been used to isolate clones from λgt11 DNA expression libraries of M. tuberculosis and M. leprae. The sequence of the DNA clones revealed that mycobacterial hsp70 (alias 70 kDa antigen) and hsp60 (alias 65 kDa antigen, groEL) were the major targets of the murine antibody response to both M. tuberculosis and M. leprae. Two additional hsp's, an 18 kDa member of the small hsp family and a 12 kDa homologue of groES, were found among the M. leprae and M. tuberculosis antigens. Young, D.B., et al., Proc. Natl. Acad. Sci. USA, 85:4267-4270 (1988); Shinnick, T.M., et al., Nuc. Acids Res., 17:1254 (1989).

suffering from malaria, trypanosomiasis, leishmaniasis, shistosomiasis and filariasis. Hsp90 is also a target of antibodies in trypanosomiasis and a member of the small hsp family is recognized in some patients with shistosomiasis.

Stress Proteins and Autoimmune Processes

Rheumatoid arthritis is characterized by a chronic proliferative and inflammatory reaction in synovial membranes which is thought to involve autoimmune processes. Rat adjuvant arthritis resembles human rheumatoid arthritis in many respects, and has been used as an experimental animal model for human disease. Pearson, C.M., Arthritis Rheum., 7:80-86 ((1964). Adjuvant arthritis can be induced in rats with a single intradermal injection of killed M. tuberculosis in complete Freund's adjuvant. An autoimmune process involving T lymphocytes appears to be responsible for the generation of the disease. Holoshitz, J., et al., Science, 219:56-58 (198^). T cell lines isolated from the draining lymph nodes of arthritic rats and propagated in vitro by stimulation with M. tuberculosis-pulsed syngeneic antigen presenting cells can cause a transient form of the disease when transferred to irradiated rats. Since care was taken in these experiments to exclude the transfer of contaminating M. tuberculosis, this result strongly suggests that the clinical effects of the disease are a consequence of an autoimmune reaction in which the autoantigen is shared with M. tuberculosis.

The rat and M. tuberculosis antigens recognized by the arthritogenic T cells have been sought for a number

of years. A number of different proteins present in synovial membranes have been proposed to be the cross-reactive rat antigen, but were later discounted as procedures for the purification of these proteins improved. van Eden, W., et al., Proc. Natl. Acad. Sci., USA, 82:5117-5120 (1985); Holoshitz, J., et al., Science, 219:56-58 (1983). The M. tuberculosis antigen recognized by the arthritogenic T cells was recently shown to be a 65 kDa protein (van Eden, W., et al., Nature, 331:171 (1988), which has now been shown to be hsp60 (see the Exemplification). Using a combination of truncated recombinant 65 kDa proteins and peptides, a nine amino acid epitope of hsp60 has been identified as the minimum stimulatory sequence for arthritogenic T cell clones in proliferation assays. Now that it is clear that some arthritogenic T cells recognize the mycobacterial hsp60, it is quite possible that the rat autoantigen is also hsp60.

The results obtained in the adjuvant arthritis model led investigators to determine whether T lymphocytes from human rheumatoid arthritis patients also recognize mycobacterial antigens. These investigators have found not only that patients with rheumatoid arthritis have T cells that recognize M. tuberculosis antigens, but that these T cells have diverse phenotypes. Substantial proliferative responses to mycobacterial extracts are observed with uncloned T cells (predominantly $CD4^+$) from both synovial infiltrates and peripheral blood, although responses are generally greater in synovial infiltrates. Abrahamson, T.G., et al., Scand. J. Immunol., 7:81-90 (1978); Holoshitz, J., et al., Lancet ii, 305-306 (1986).

Holoshitz et al. found that 4 of 5 T cell clones isolated from human rheumatoid synovia which respond to M. tuberculosis antigens were $CD4^-$ $CD8^-$ cells with γ/δ T cell receptors. Holoshitz, J., et al., Nature, 339:226-229 (1989). This observation is interesting because γ/δ T cells have yet to be assigned a role in immunity. One of the γ/δ clones was tested for its ability to respond to purified mycobacterial hsp60 and was found to be positive in proliferation assays. Due to the conserved nature of stress proteins, these T cells have the potential for autoreactivity. Lamb and coworkers have shown that polyclonal T cells from synovial infiltrates recognize both mycobacterial hsp60 and hsp70. Lamb, J.R., et al., Intl. Immunol., in press (1989). The population of T cells that recognize the mycobacterial stress proteins were shown to respond to E. coli hsp60 and hsp70 and, most interestingly, human hsp70 purified from heat shocked macrophages. Thus, immune responses to conserved stress protein determinants, perhaps initiated by bacterial infection (not necessarily by mycobacteria), may play an important role in autoimmune pathology in rheumatoid arthritis, as well as in adjuvant arthritis.

Stress Proteins and Immune Surveillance

A variety of different T cell types has now been shown to recognize highly conserved stress protein determinants. The ability of cells to respond to stress by increasing the levels of the highly conserved stress proteins; the presence of T cells of diverse phenotypes in healthy individuals that are capable of recognizing

CLAIMS

1. A vaccine comprising all or a portion of a selected stress protein or all or a portion of a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of the stress protein.

2. A vaccine of Claim 1 in which the stress protein is a mycobacterial stress protein or a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of the mycobacterial stress protein.

3. A composition for use as an agent to induce immune tolerance, comprising a selected stress protein.

4. A composition for use in treating an autoimmune disease, comprising all or a portion of a selected stress protein or all or a portion of a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of the stress protein.

5. A composition of Claim 4 for treating rheumatoid arthritis.

6. A vaccine for use in enhancing in an individual the immune response to a nonviral pathogen, comprising all or a portion of a stress protein of the nonviral pathogen against which the enhanced response is desired.

7. A vaccine of Claim 6 in which the stress protein is a mycobacterial stress protein.

8. A method of producing or enhancing an immune response in an animal, comprising administering to the animal a selected stress protein, in sufficient quantity to elicit the desired immune response.

9. A method of immunizing an animal against a subsequent nonviral infection, comprising administering to the animal a selected stress protein, in sufficient quantity to produce an immune response.

10. A method of inducing in an individual immune tolerance against a protein, comprising administering to the individual all or a portion of a selected stress protein or all or a portion of a protein having an amino acid sequence sufficiently homologous to the amino acid sequence of the stress protein, under conditions appropriate for induction of the desired tolerance.

11. A method of Claim 10, wherein the protein is a protein associated with rheumatoid arthritis.